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EXPLORING THE POTENTIAL BIODEGRADATION OF SELECTED PERFLUOROALKYL CARBOXYLIC ACIDS IN BIOLOGICALLY ACTIVE WASTEWATER

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants that can enter municipal wastewater from domestic and industrial sources. Perfluoroalkyl carboxylic acids (PFCAs) are especially difficult to remove because of the high stability of the carbon-fluorine bond. Biological treatment may still contribute to the removal of these compounds. PFAS can interact with microbial biomass through biosorption, cellular uptake, partitioning, or possible biotransformation. For this reason, microorganisms present in wastewater treatment systems may play a role in the fate of PFAS. This study focused on four PFCAs with different carbon-chain lengths: perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA). Two bacterial strains isolated from municipal wastewater were tested: the Gram-negative *Klebsiella oxytoca* and the Gram-positive *Bacillus cereus*. The aim was to evaluate the decrease in PFAS concentration during bacterial exposure and to assess possible effects on bacterial growth.

Materials and methods

Klebsiella oxytoca (B1) and *Bacillus cereus* (B2) were isolated from municipal wastewater. The strains were first grown on solid nutrient medium for 24 h at 35°C. One colony from each strain was then transferred to tryptic soy broth (TSB) and incubated for 24 h at 37°C and 130 rpm. Bacterial growth was measured at 600 nm, and cultures with an OD_{600} of approximately 0.2 were used in the experiments. Each strain was separately exposed to PFOA, PFNA, PFDA, and PFUnDA at 0.1 mg/L for 96 h. Samples were collected at 0, 2, 4, 24, 48, and 96 h. The experimental design is summarized in Figure 1. Residual PFAS concentrations were determined by LC-MS/MS, while bacterial growth was monitored spectrophotometrically at 600 nm. Positive controls contained the bacterial strain in TSB without PFAS, while negative controls consisted of uninoculated medium containing the corresponding PFAS. Apparent removal was calculated relative to the initial PFAS concentration. Since concentration decrease may also result from biosorption, biomass partitioning, or cellular uptake, the observed effect was considered apparent biotic removal rather than confirmed biodegradation.

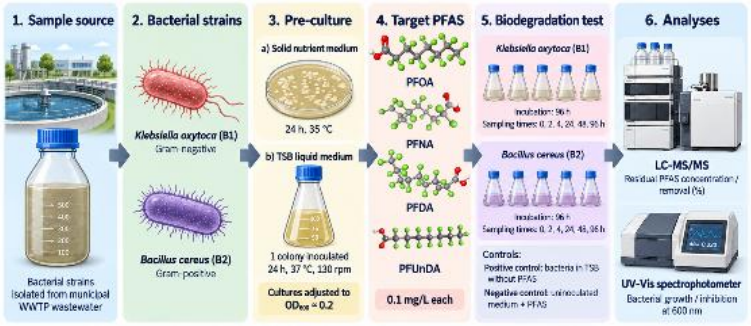


Figure 1 - Experimental design used to evaluate the potential biodegradation of selected PFCAs by *K. oxytoca* (B1) and *B. cereus* (B2)

Results and conclusions

Both bacterial strains remained active during the 96h exposure period. The concentrations of the tested PFCAs decreased over time, but the behavior differed between compounds and bacterial strains. *K. oxytoca* showed moderate removal for all tested PFCAs. The response was relatively uniform. PFNA and PFDA showed a more pronounced decrease, while PFOA and PFUnDA showed lower removal. A different pattern was observed for *B. cereus*. The response was more dependent on the PFCA tested. PFDA showed the highest apparent removal during the experiment. PFOA was only slightly affected. PFNA and PFUnDA showed intermediate behavior. These differences suggest that the interaction between PFAS and bacterial biomass may depend on the chemical properties of each compound. Carbon-chain length may be one of the factors involved. The tested PFAS caused only limited inhibition of bacterial growth. In most cases, the effects were low and temporary. Both bacterial strains therefore remained biologically active during exposure.

The results show that bacterial strain and PFCA type can influence PFAS removal under biological conditions. *K. oxytoca* showed a moderate and relatively similar response for the four compounds. *B. cereus* showed a more selective behavior, with the strongest effect observed for PFDA. However, the decrease in PFAS concentration alone cannot confirm biodegradation. Further studies are needed to distinguish true biotransformation from biosorption or partitioning to biomass.

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